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The Ultrastructure of Bovine Cervical Mucus under Scanning Electron Microscope

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Summary

The cervical mucus of cows during estrous cycle was observed under scanning electron microscope.

During estrus it consisted of a network with entangled filaments of two different sizes. The large filaments appeared to support a main structure and the samll ones exhibited a cobweb-like distribution.

The mucus during diestrus showed a tightly-closed mesh.

Spermatozoa were able to invade the network of mucus in estrus, and in a mucus sample in estrus leucocytes were adhered to the spermatozoa.

It is known that in the cow, cervical mucus acts as a barrier which is only permeable to the spermatozoa about the time of ovulation (1).

It's chemical composition and physical properties have been intensively studied (2, 3) but relatively little data is available concerning the structure because of difficulty of studying mucoid samples with light microscopy.

However, scanning electron microscopy (SEM) has made it possible to study the three- dimentional structure of the mucus of humans (4).

The purpose of this study is to investigate the ultrastructure of cervical mucus of cows during estrous cycle under the scanning electron microscope.

Materials and Methods

Eleven cows of Hostein breed, reared in the Tohoku University Farm, were used to collect samples. Six of them were artificially inseminated with frozen bull semen on the day of standing estrus. The samples were collected from ten cows in estrus and one cow in luteal stage. A vinyl tube, 6 mm in internal diameter, was inserted into the cervix to aspirate the mucus.

One of the samples was immediately fixed in 2.5% glutaraldehyde (0.1 M phosphate buffer, pH 7.4). Following dehydration in alcohol, the critical drying point in liquid CO_2 and the gold coating for the scanning microscopic observation

using a FE type electron microscope (HITACHI S–700) was performed. Other samples were smeared on a glass slide and air dried, followed by staining with Alcian blue & Kernechtrot for light microscopic observation.

Results

1. Observation of bovine cervical mucus

In estrus, the cervical mucus was observed as a network of entangled filaments by SEM (Plate 1–1, 2).

The large filaments appeared to be the main structural supports of mucus, whereas the small ones formed a complex network structure (Plate 2–1).

The large filaments were arranged in parallel and were linked by very small traversing filaments (Plate 2–2).

The small ones were entangled (Plate 2–3) and showed a cobweb-like distribution (Plate 2–4). Channels which gave the impression of a three-dimensional structure running through the mucus were seen in a network structure (Plate 1–3).

In diestrus, the mucus exhibited a tightly-closed mesh (Plate 2–5) with a decrease in the size of mesh (Plate 2–6).

The types of filament arrangements were observed to be different among each sample of mucus in estrus, and in addition, the frequency of filaments types was quite variable among different areas of a single sample.

2. Observation of spermatozoa in the bovine cervical mucus

The sperm head appeared to destroy the structure of network (Plate 3–1), and then to invade the mucus (Plate 3–2, 3, 4). Sperm were observed in the gap between the large filaments (Plate 3–5) and a spermatozoon lay on the surface of mucus partly covered by filaments (Plate 3–6). Sperm tail and head will be available for estimation of the relative size of the mucus filament and network.

3. Observation of leucocyte in bovine cervical mucus

A large number of leucocytes, identified as neutrophil, were observed in one of eleven mucus samples in estrus by both light microscope (Plate 4–1) and SEM.

They were distributed widely in the network structure (Plate 4–2) and some of them were adherred to the sperm head and tail (Plate 4–3, 4, 5).

Discussion

The present results obtained by scanning electron microscopic observation clearly showed that bovine cervical mucus consisted of a network of entangled filaments, which corresponded with the observation by transmission electron microscopy (5).

This is also in agreement with the observation of human (4) and baboon (6)

cervical mucus. We observed in the present experiment that the arrangement of filaments was different among each sample of mucus in estrus. Moreover, the frequency of filament's types was quite variable among different areas of a single sample.

Thus, we suppose that the bovine cervical mucus may be a delicate material with physical and chemical property variable even during estrous period.

Another fact known is that the mucus in estrus consists of 95 to 98% water, and the apparent degree of packing of filaments in mucus is dependent upon the procedure of preparation of alchohol dehydration: namely the higher the water content, the greater the mucus is dehydrated.

Channels could have been present on the surface of mucus, but they were not large enough for a sperm head to easily pass through.

Sperm head invaded the mucus, although it is uncertain whether those spermatozoa would imigrate to the uterus through the cervical mucus. Leococyt's attachment to spermatozoa probably leads to phagocytosis of them (7–11).

Our observations suggest that the network structure of mucus in estrus may play a role of discriminating weak sperm from strong ones which are available for fertilization.

They suggest that the filaments of mucus in estrus may be in sol state which is soft enough for sperm to easily cut through or break.

It is thought that the large filaments align in parallel within the cervical lumen and that this distribution of mucus is a prerequiste to the passage of spermatozoa through the mucus to the uterus.

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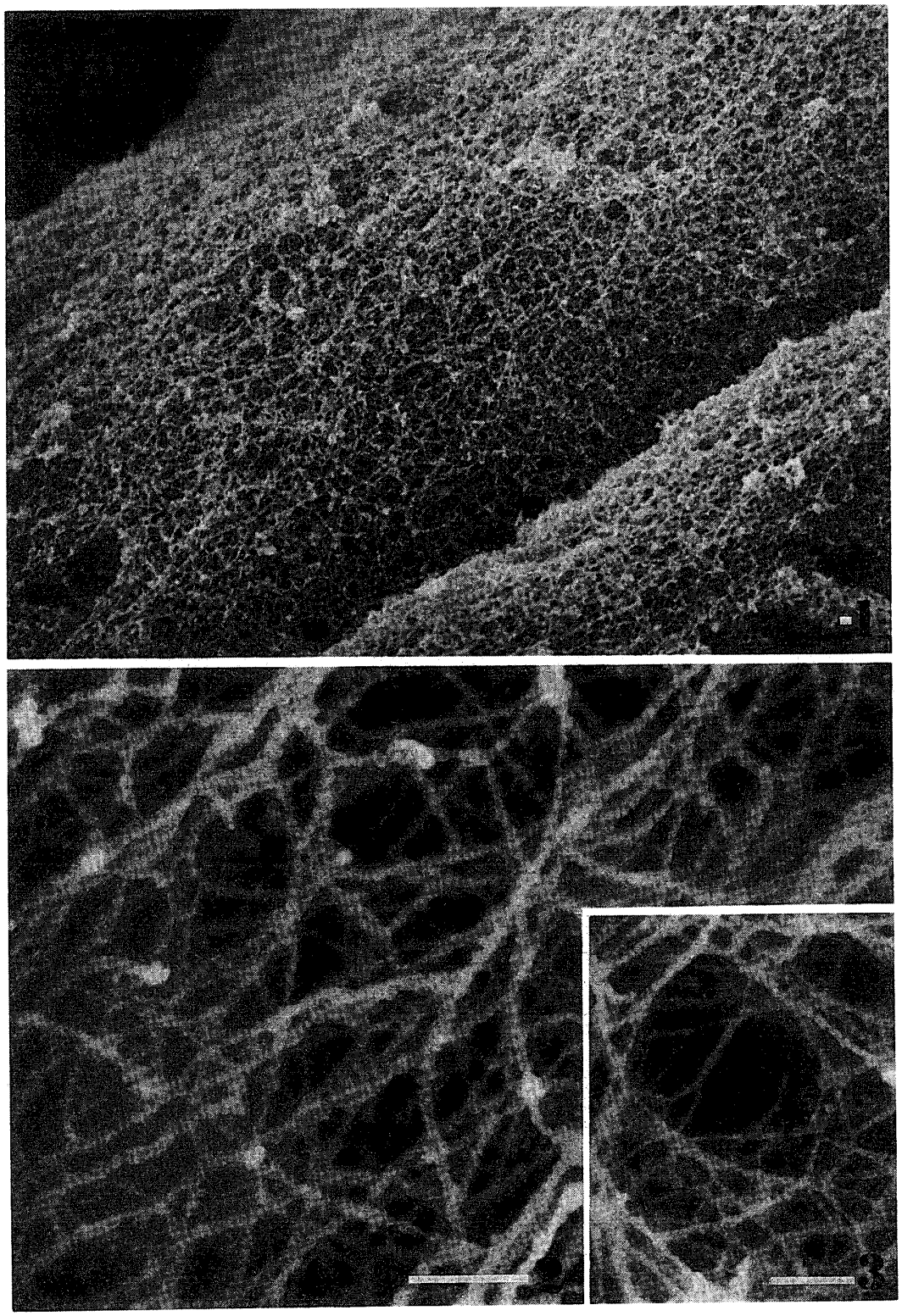

PLATE 1. Bovine cervical mucus in estrus. Scale indicates 1 μm.
FIGS. 1 & 2. Cervical mucus consisting of a network of entangled filaments.
FIG. 3. Channel of a three-dimentional structure running in the mucus.

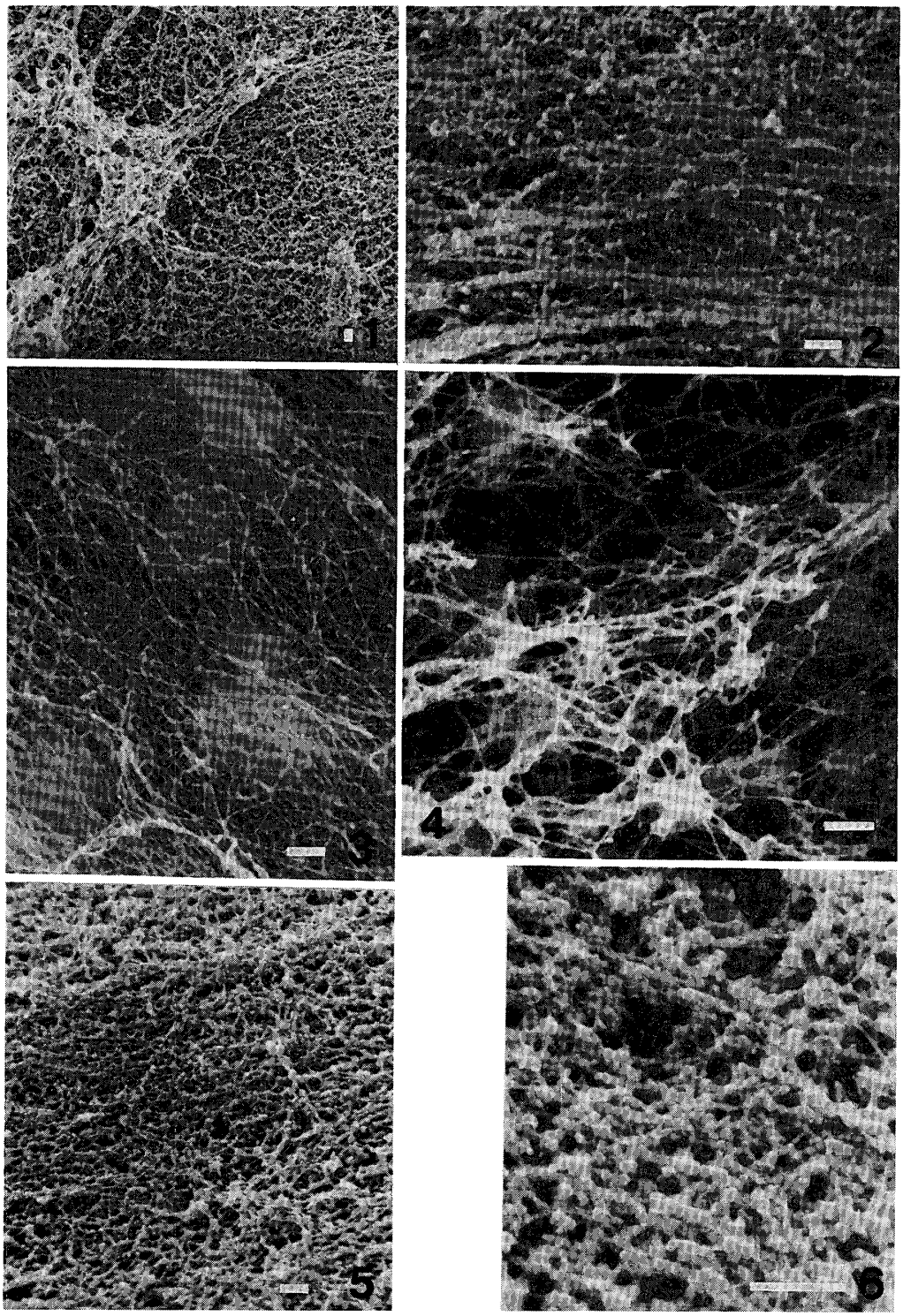

PLATE 2. Bovine cervical mucus in estrus and diestrus. Scale indicates 1 μm.

FIG. 1. At estrus. Filaments of two sizes were observed. The large filaments appear to support the mucus structure.

FIG. 2. At estrus. The large filaments arranged in parallel and linked by traversing small filaments.

FIG. 3. At estrus. The small filaments entangled like a network.

FIG. 4. At estrus. Small filaments of cobweb-like distribution.

FIG. 5. At diestrus. The surface of mucus with a tightly closed mesh.

FIG. 6. At diestrus. The mesh of decreased size.

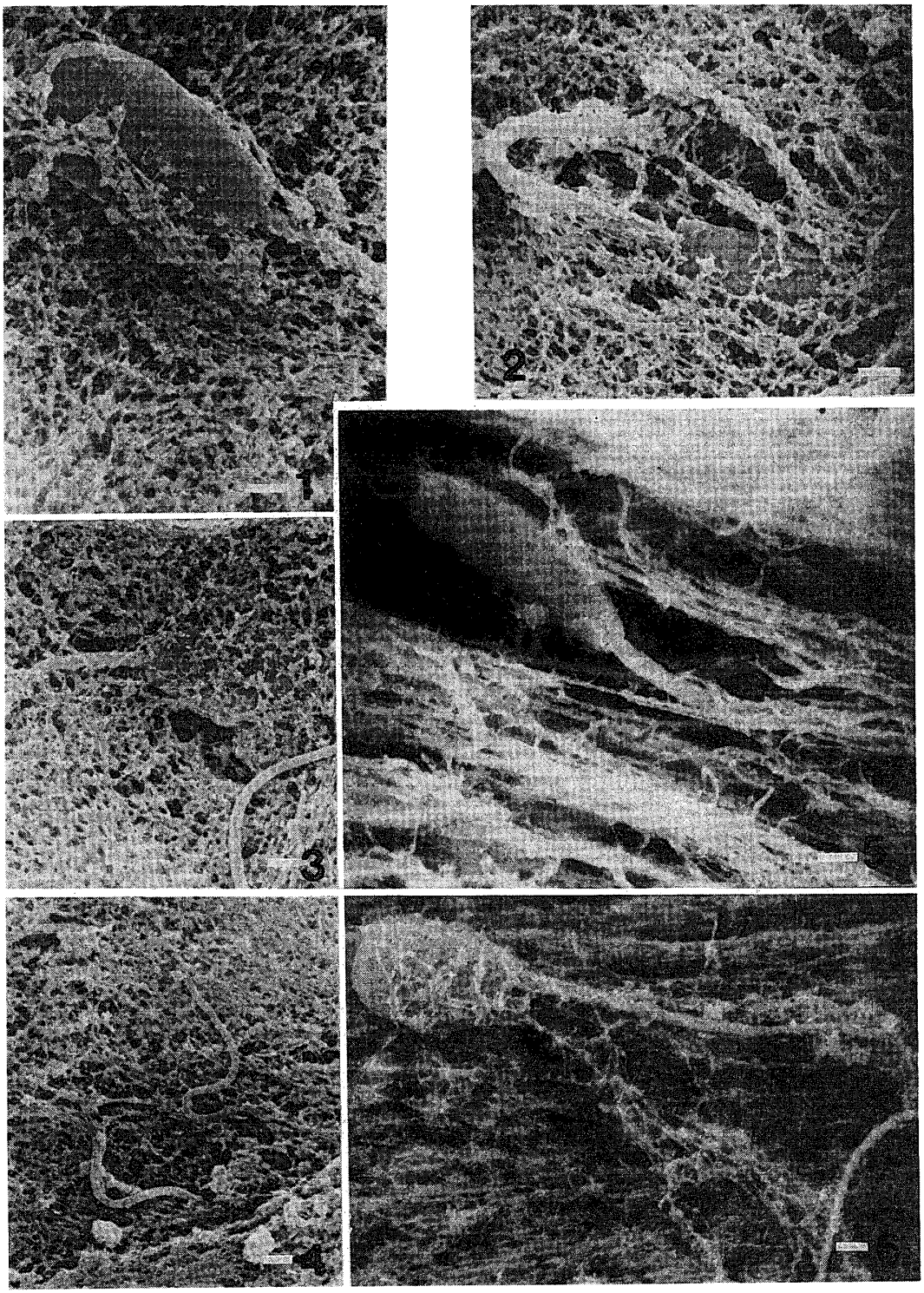

PLATE 3. Spermatozoa in bovine cervical mucus. Scale indicates 1 μm.
FIGS. 1–4. Spermatozoon invading the mucus.
FIG. 5. A spermatozoon in a space of the mucus.
FIG. 6. A spermatozoon lying on the surface of mucus, trapped within mucus filaments.

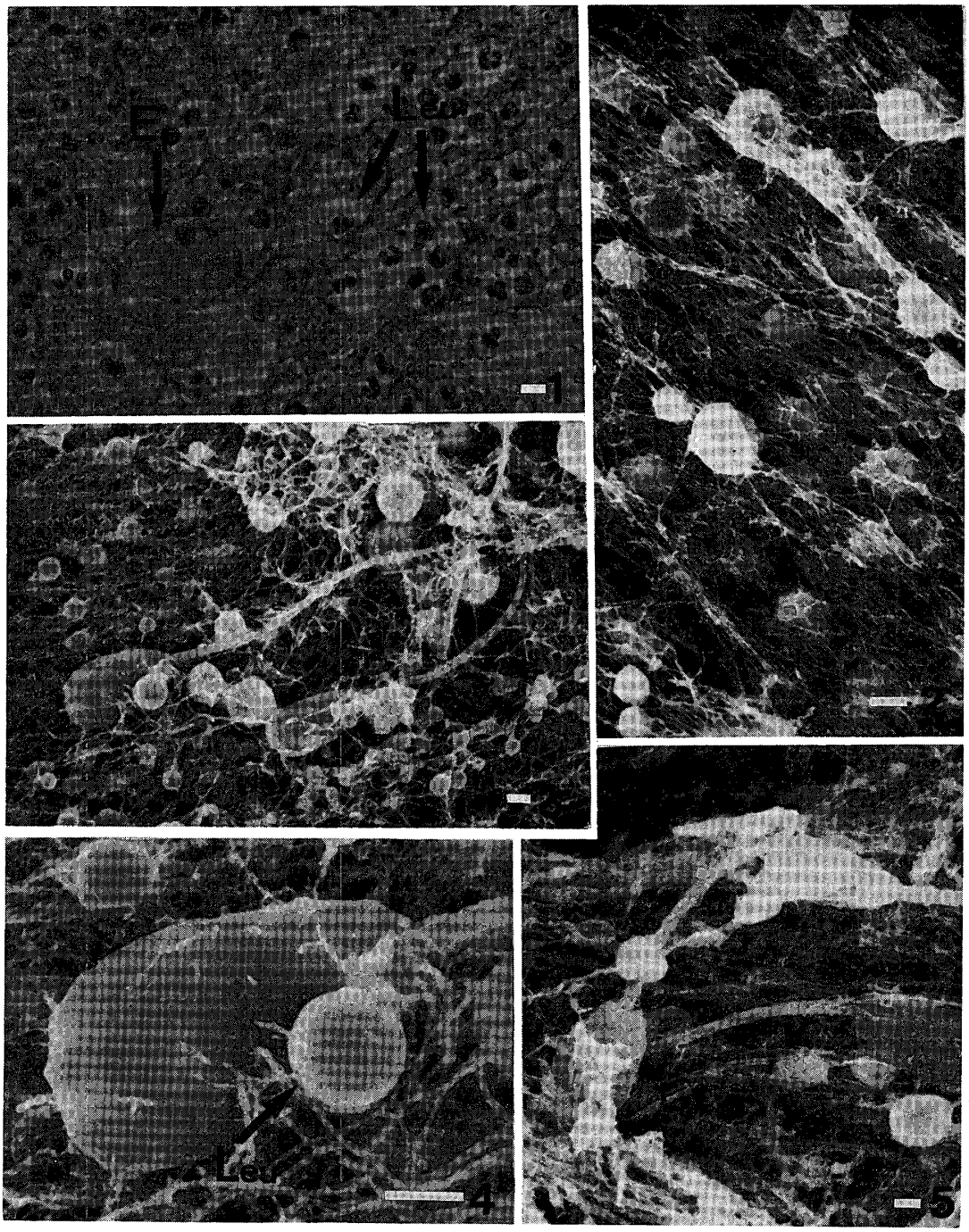

PLATE 4. Leucocytes in bovine cervical mucus. Scale indicates 1 μm.

FIG. 1. Neutrophil leucocytes in mucus (by light microscopy, Alcian blue & Kernechtrot stain).
Ep: Epithelial cell, Leu: Neutrophil leucocytes.

FIG. 2. Leucocytes distributed in mucus (SEM).

FIG. 3. Leucocytes adhered to sperm head and tail (SEM).

FIG. 4. Magnification of Fig. 3 (SEM).

FIG. 5. Leucocyte adhered to sperm neck (SEM).